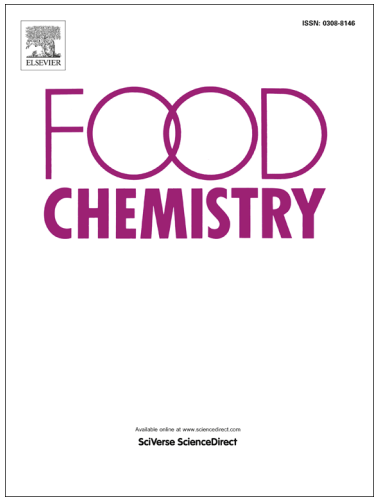
CORE
Metadata, citation and similar papers at core.ac.uk
Provided by CONICET Digital

Accepted Manuscript



Optimization of β-cyclodextrin-based extraction of antioxidant and anti-browning activities from thyme leaves by response surface methodology

Leonardo Cristian Favre, Cristina dos Santos, María Paula López-Fernández, María Florencia Mazzobre, María del Pilar Buera

PII: S0308-8146(18)30889-6
DOI: https://doi.org/10.1016/j.foodchem.2018.05.078
Reference: FOCH 22911

To appear in: *Food Chemistry*

Received Date: 3 March 2018
Revised Date: 15 May 2018
Accepted Date: 16 May 2018

Please cite this article as: Favre, L.C., dos Santos, C., López-Fernández, M.P., Mazzobre, M.F., Buera, M.d.P., Optimization of β-cyclodextrin-based extraction of antioxidant and anti-browning activities from thyme leaves by response surface methodology, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.05.078

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Optimization of β-cyclodextrin-based extraction of antioxidant and anti-browning activities from thyme leaves by response surface methodology

Leonardo Cristian Favre [1 2]; Cristina dos Santos [1]; María Paula López-Fernández [2 3 4]; María Florencia Mazzobre [1 2]; María del Pilar Buera [1 2 *]

[1] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamentos de Industrias y Departamento de Química Orgánica. Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Buenos Aires, Argentina.

[2] CONICET - Consejo Nacional de Investigaciones Científicas y Técnicas. Godoy Cruz 2290, C1425FQB, Buenos Aires, Argentina.

[3] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, DBBE - Departamento de Biodiversidad y Biología Experimental. Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Buenos Aires, Argentina.

[4] CONICET - Consejo Nacional de Investigaciones Científicas Técnicas, IBBEA - Instituto de Biodiversidad y Biología Experimental y Aplicada. Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Buenos Aires, Argentina.

* Corresponding author: María del Pilar Buera, pilar.buera@gmail.com

Phone: (+54) (+11) 5284 9037/36/18

Keywords: Thyme; β-Ciclodextrin; Anti-glycation; Maillard; Antioxidant activity; Ultrasonic.

Abstract

Thyme (*Thymus vulgaris*) has been demonstrated to extend the shelf-life of food products, being also a potential source of bioactive compounds. The aim of this research was to optimize the ultrasound assisted extraction employing β-cyclodextrin aqueous solutions as no-contaminant technology and Response Surface Methodology to obtain thyme extracts with the maximum antioxidant capacity. The optimal extraction conditions were: a solution of β-ciclodextrin 15mM, an ultrasonic treatment time of 5.9 min at a temperature of 36.6°C. They resulted in an extract with a polyphenolic content of 189.3 mg GAE/mL,

ACCEPTED MANUSCRIPT

an antioxidant activity (DPPH•) of 14.8 mg GAE/mL, and ferric reducing/antioxidant power (FRAP) of 3.3 mg GAE/mL. Interestingly, the extract demonstrated to inhibit the production of Maillard browning products and can be considered a potential antiglycant agent. The obtained data is important for developing eco-friendly technologies in order to obtain natural antioxidant extracts with a potential inhibitory capacity of Maillard glycation reaction.

Chemical compounds

β-Cyclodextrin (β-CD) (PubChem CID: 444041); Bovine serum albumin (BSA) (PubChem CID: 16132389); Glucose (PubChem CID: 5793); 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) (PubChem CDI: 2735032); 2,4,6-Tripyridyl-s-triazine (TPTZ) (PubChem CID: 77258); Gallic acid (PubChem CID: 370); Ferric chloride hexahydrate (PubChem CID: 6093258); Phosphate buffered saline (PBS) (PubChem CID: 24978514); Sodium carbonate trihydrate (PubChem CID: 10340).

1. Introduction

Since ancient times, spices are considered natural flavouring and preservatives agents to protect food components from deterioration (Wojdyło, Oszmiański, & Czemerys, 2007; Nipornram, Tochampa, Rattanatraiwong, & Singanusong, 2018; Rakmai, Cheirsilp, Mejuto, Torrado-Agrasar, & Simal-Gándara, 2017). Among the most important spices with antioxidant properties, plants from the Lamiaceae family (oregano, thyme, sage, marjoram, basil, coriander, and pimento) are predominant (Torre et al., 2017). In recent studies Thyme (*Thymus vulgaris*), a bush native from the western Mediterranean region, was found to possess efficient antimicrobial and antifungal activities and is used in some foods to extend the shelf-life (Assiri, Elbanna, Abulreesh, & Ramadan, 2016a; Liu et al., 2017).

Non-enzymatic browning (mainly Maillard) and oxidative reactions are a constant concern in food industry. The research on the potential inhibitory compounds and the antioxidants obtained from edible vegetables is growing since they are non-toxic and have no known adverse side effects (Balasundram, Sundram, & Samman, 2006). Sulphites, consider browning universal inhibitors and synthetic antioxidants compounds, have been employed for decades for controlling enzymatic and non-enzymatic browning and lipid deterioration. Since the use of sulphites have been banned in fresh fruits and vegetables (Food and Drug Administration, 1987) and the synthetics antioxidants have been questioned for its toxicity, there is a need to find, natural extracts to replace them (Martín-diana, Rico, & Barry-ryan, 2008).

ACCEPTED MANUSCRIPT

The extraction of bioactive compounds from natural sources, using green techniques and appropriate extraction solvents, constitutes an important step in the manufacturing of phytochemical-rich products with anti-browning and antioxidants properties (Gao et al., 2016). The extraction from natural sources traditionally involved the use of organic solvents, being not environmentally friendly, time-consuming and having low extraction efficiencies (Barba, Zhu, Koubaa, Sant'Ana, & Orlien, 2016). Among non-conventional extraction techniques recently reported, the ultrasonic assisted extractions (UAE) have shown higher extraction efficiency with less energy and low solvent consumption. As a result the use of ultrasound is being increasingly applied with great success (Nipornram, Tochampa, Rattanatraiwong, & Singanusong, 2018; Wakte et al., 2011). One important fact in the use of UAE is the selection of the extraction solvent. Although organic solvents do increase the extraction yield, they are associated with environmental pollution (Chemat, Vian, & Cravotto, 2012). Based on this fact, extraction of natural bioactive compounds might be associated with the selection of new and green extraction solvents.

On the other hand, cyclodextrins (CDs) are cyclic oligosaccharides, typically containing six (α-CD), seven (β-CD), or eight (γ-CD) glucopyranose units with a truncated cone spacial geometry. These molecules and their derivatives such as hydroxypropyl CDs form host-guest inclusion complexes with a wide range of compounds (Rakmai, Cheirsilp, Mejuto, Simal-Gándara, & Torrado-Agrasar, 2018; Szente & Szejtli, 2004). The physicochemical properties of the encapsulated compound changes, improving its solubility, stability and/or bioavailability (Astray, Mejuto, Morales, Rial-Otero, & Simal-Gándara, 2010; Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009). CDs are widely used in food, pharmaceutical and chemical industries for improving solubility and stability of many compounds and the selectivity of reactions, as well as for separation and purification operations (Astray et al., 2009). CDs also improve the extraction of natural compounds from plant matrices. Moreover, extraction of some phenolic compounds from plants with different aqueous CDs solution has been demonstrated to be an efficient and eco-friendly extraction process (Parmar, Sharma, & Rupasinghe, 2015; Ratnasooriya & Rupasinghe, 2012). CDs have lower toxicity and less potential to produce secondary pollutants than traditional solvents being environmentally friendly additives for rapid and effective extraction of hydrophilic and hydrophobic compounds of natural sources. Technologies using CDs as extracting agents have yet to be developed in order to achieve greater yield for safe industrial applications (Gao et al., 2016; Parmar et al., 2015).

ACCEPTED MANUSCRIPT

The optimization of extraction process involves a great number of factors. Response surface methodology (RSM), a collection of statistical and mathematical techniques, is useful for development, improvement, and optimization of product and process (Deepak et al., 2008). The most extensive applications of RSM are particularly useful when several input variables have potentially influence on some performance measures or quality characteristics of the product / process.

In this context, the aim of this work was to optimize the use of aqueous β-cyclodextrin (β-CD) solutions in the ultrasonic assisted extraction of antioxidant compounds from thyme by means of Response Surface Methodology. The inhibitory capacity of the Maillard browning development of the obtained extract under optimized conditions was also assessed.

2. Materials and Methods

2.1. Materials and reagents

Dehydrated thyme leaves (*Thymus vulgaris*), were bought in the local market of Buenos Aires, Argentina. Folin-Ciocalteu (FC) reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), bovine serum albumin (BSA), ferric chloride hexahydrate and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). β-Cyclodextrin (β-CD) (containing 8 water molecules/molecule of β-CD, Mr. 1135) was obtained from Roquette-Food, France. Analytical grade sodium acetate, sodium carbonate trihydrate, hydrochloric acid (12 M) and ethanol were purchased from Biopack, Buenos Aires, Argentina.

2.2. Ultrasound-assisted extraction

Thyme leaves were grounded to fine powder (about 10 µ particle size) in a Butt mill, mixed with B-CD aqueous solution in a plastic tube (1:4 w/w) and submitted to ultrasound-assisted extraction in an ultrasonic UP100H (Hielscher Ultrasonics GmbH, Germany) equipped with a sonotrode MS2 for different times. An acoustic power density 600 W/cm^2 (0.5 cycle), amplitude de 220 µm (100 %) was provided. The independent variables i.e. ultrasonic treatment time (from 0.5 to 15 min), B-CD concentration (0-15 mM) and extraction temperature (20-50 °C) were considered. The thyme extracts were centrifuged (30 min, 6372 rcf, 4 °C) and the supernatant was collected for analysis.

2.3. Experimental design

The extraction conditions were optimized through a Box–Behnken design (BBD). Independent variables (X) and their levels were: β-CD concentration in a range of 0 and 15 mM, ultrasonic treatment 0-15 min

ACCEPTED MANUSCRIPT

and extraction temperature 20-50 °C. The responses were evaluated through the BBD experiment by the following dependent variables (Y): total polyphenolic contents (TPC), ferric reducing/antioxidant power (FRAP) and the antiradical capacity as the degradation of the radical DPPH•, which were codified as shown in Table 1.

The predicted responses were calculated by a second-order polynomial model shown in Equation 1:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} b_{ij} X_i Y_j \quad (1)$$

where Y is the response value, b_0 is the offset term, b_i is the linear effect, b_{ii} is the squared effect, b_{ij} is the interaction effect and X_i and X_j are the independent variables.

The desirability function D (Eq. 2) allows multiple response optimization to find the extraction conditions leading to the commitment levels of the analyzed variables (Derringer, G. & Suich, 1980).

Desirability was obtained using Equation 2:

$$D = (d_1(Y_1) \cdot d_2(Y_2) \dots d_i(Y_i))^{1/i} \quad (2)$$

where d_i (Y_i) are the normalized values (from 0 to 1) of each studied response.

The data and coefficients were analyzed using the Design-Expert® software (10 version) and the *F*-value, respectively. The experimental conditions of the dependent variables were performed applying an analysis of variance (ANOVA), regression analysis and plotting of RSM figures.

2.4. Antioxidant activity assays

β-CD can encapsulate the reagents involved in the determinations of antioxidant activity (FC, DPPH• and FRAP) (Schlesier, Harwat, Böhm, & Bitsch, 2002), controls were prepared adding water, β-CD solutions (7.5 mM and 15 mM) according to the corresponding system, in order to subtract the effects of the matrix at each condition of the experimental design.

2.4.1. Total polyphenolic contents by Folin-Ciocalteu (FC)

Total polyphenolic contents of the thyme extracts were determined using Folin-Ciocalteu method according to Singleton et al. (1999) with modifications. An aliquot (50 µL) of thyme extract sample, 800 µL distilled water, 125 µL sodium carbonate solution (20 % w/v) and 125 µL FC reagent (1M) were

ACCEPTED MANUSCRIPT

mixed. After 30 min at 30 ºC, the absorbance of the solutions was measured at 765 nm. The total polyphenolic contents were expressed as mg of gallic acid equivalent (GAE) per mL thyme extract.

2.4.2. Ferric reducing/antioxidant power (FRAP)

The ferric reducing/antioxidant power of the samples was determinate according to Benzie & Strain (1996). The FRAP reagent was composed of 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, plus 2.5 mL 20 mM $FeCl_3.6H_20$ and 25 mL 0.3 M acetate buffer at pH 3.6. An aliquot of 840 µL of the FRAP reagent, freshly prepared was mixed with 60 µL of each sample and after 30 min at 37 ºC, the absorbance of the solutions was measured at 595 nm. The ferric reducing/antioxidant power was expressed as mg of GAE per mL thyme extract.

2.4.3. Free radical scavenging by DPPH• assay

The reaction mixture was prepared adding 50 µL of each thyme extract sample to 950 µL of an ethanolic solution of DPPH• radical with an initial absorbance equal to 1 at 517 nm (Chaillou & Nazareno, 2006). The absorbance at 517 nm was determined after 30 min of reaction. The percentage of DPPH• radical inhibition was calculated according to the Equation 3:

$$\%AAR = 100 \times \left[1 - \frac{A_{SS}}{A_0}\right] \quad (3)$$

where A_{SS} is the absorbance of each solution after 30 min of reaction and A_0 is the absorbance of DPPH• control solution. The results were expressed as mg of GAE per mL thyme extract.

2.5. Validation of the model

The optimized values of the independent variables, selected from the maximum extraction conditions predicted using the D function, permitted to obtain the experimental response data (TPC, antioxidant DPPH• activity, antioxidant/reducing power (FRAP assay)). In order to validate the applied model these data were compared with the predicted values based on percentage difference (PD) calculated using Equation 4:

$$\mathrm{PD} = \frac{\Delta X}{\overline{X}} \times 100 \quad (4)$$

where ΔX is the difference between the predicted and the experimental value, and $\overline{X}$ is the mean of experimental response.

ACCEPTED MANUSCRIPT

2.6. Anti-glycant capacity studying the UV-Vis absorbance and browning of Maillard products

As reported in previous works, the study of anti-glycant capacity of the thyme extract (ThyE) obtained under optimized conditions was performed by analyzing the inhibition of Maillard reaction in model systems containing bovine serum albumin (BSA) + glucose (GLU), BSA+GLU+β-CD and of BSA+GLU+ThyE (3 % v/v) and measuring the absorbance at 420 nm at selected reaction times (Morimitsu, Yoshida, Esaki, & Hirota, 1995).

BSA and GLU were dissolved in sodium phosphate buffered saline (PBS) (0.2 M, pH 7.40) to obtain 10 mg/mL and 50 mg/mL solutions, respectively. The ThyE was obtained in the experimental conditions predicted by the RSM previously studied. After adding the ThyE, the pH of the solutions was measured with a pH meter (Mettler Toledo 340, USA), and adjusted to a pH 7.40. The reaction systems were placed in glass vials, sealed and stored at 70, 80 and 90 °C during 0, 30, 60, 120, 240 and 360 min in a bath with circulating water. Finally, the reaction was stopped by cooling the vials at 4 ºC.

The UV–Visible absorption of the systems at 420 nm was measured at room temperature in a spectrophotometer Jasco V-630 Uv-Vis (JASCO Inc., Easton, MD, USA) after storage at the different assayed conditions (Zhou, Li, & Yu, 2016). Triplicate analyses of each sample were performed, and the mean value and standard deviation were used to construct kinetic plots.

2.7. Browning kinetics

The reaction rate constants (k) of browning products (BPs) formation were calculated from the straight-line plot of the systems (BSA+GLU; BSA+GLU+β-CD) absorbance at 420 nm in presence and absence of 3 % v/v of ThyE, versus the reaction time for each assayed temperature. The Arrhenius equation ($\ln k = \ln k_0 - E_a/RT$) was used to analyze the effect of temperature and composition on the Maillard reaction kinetic. Activation energy (E_a) of each system was calculated from the slope of the Arrhenius equation representation ($\ln k$ versus 1/T).

2.8. Statistical analysis

All experiments were carried out in triplicates. Means and standard deviations of the data were calculated for each treatment. Analysis of variance (ANOVA) was carried out to determine any significant differences ($p<0.05$). The curve fitting, kinetics calculation and correlation analysis were performed using GraphPad Prism 6.0 software (San Diego, California, USA). Pearson correlation coefficients and p-values were used to show correlations and their significance. All statistical analyses were carried out at a 95 % level of confidence.

ACCEPTED MANUSCRIPT

3. Results and discussion

3.1. Fitting the model

Table 1 shows the values of independent variables (β-CD concentration, ultrasonic treatment time, extraction temperature) employed in RSM for optimizing the total polyphenolic contents, ferric reducing/antioxidant power, and antioxidant activity of the thyme extract. Experiment #1 (β-CD concentration 7.5 mM, ultrasonic treatment time 7.7 min and extraction temperature 35 °C) provided the highest total polyphenolic content (199.61 mg GAE/mL) and the highest ferric reducing/antioxidant power (3.36 mg GAE/mL). Experiment #3 (β-CD concentration 0 mM, ultrasonic treatment time 7.7 min and extraction temperature 20 °C) provided the lowest polyphenolic content (126.79 mg GAE/mL). From experiment #7 (β-CD concentration 0 mM, ultrasonic treatment time 0.5 min and extraction temperature 35 °C) the highest antioxidant activity (16.14 mg GAE/mL) was obtained and experiment #11 (β-CD concentration 7.5 mM, ultrasonic treatment time 15 min and extraction temperature 20 °C) produced the lowest antioxidant activity (8.86 mg GAE/mL). Experiment #13 (β-CD concentration 7.5 mM, ultrasonic treatment time 0.5 min and extraction temperature 20 °C) produced the least ferric reducing/antioxidant power (1.04 mg GAE/mL).

The analysis of variance ANOVA presented in Table 2 showed that the experimental values of all the responses data can be fitted using a quadratic polynomial model (p-value <0.0001). The F-value 33.67, 60.16 and 235.98 for the total polyphenolic contents, the antioxidant activity and the ferric reducing/antioxidant power of the thyme extracts respectively, suggested that the significance of the model was higher than the 95% confidence level. Table 2 shows the lack of fit and the value of pure error, indicating the well reproducibility of the experimental data.

The effect of the extraction factors X_1 (β-CD concentration), X_2 (ultrasonic treatment time) and X_3 (extraction temperature) were carefully analyzed for each response factor (Table 1). The significance of each coefficient was determined by F-values and p-values, considering that higher F-value with lower p-value always led to more significant corresponding between various independent variables (Chen, Zhao, & Yu, 2015).

3.1.1. Response surface analysis of total polyphenolic contents

ACCEPTED MANUSCRIPT

The following regression equation in coded level, avoiding insignificant terms, was generated to analyze the effect of each independent variable on the total polyphenolic contents extraction from thyme extracts (Eq. 5)

$$Y_1\ (\text{TPC, mg GAE/mL}) = 181.36 + 7.63X_1 + 13.06X_3 - 16.06X_1X_3 - 11.92X_2^2 - 24.09X_3^2 \quad (5)$$

The quadratic polynomial equation of the relationship between the total polyphenolic contents and the extraction factors had a regression coefficient (R^2) of 0.8965 (Table 2). These values of R^2 are considered appropriate for this type of model (Chen et al., 2015; Nipornram et al., 2018; Rezende, Nogueira, & Narain, 2017).

The variables X_1, X_3, X_1X_3, X_2^2 and X_3^2, were significant (*p*-value less than 0.05). However, X_2, X_1X_2, X_2X_3 and X_1^2 were not significant due to a higher *p*-value (i.e. higher than 0.05). The combination of variables X_1X_3 showed a synergistic effect between the β-CD concentration and the extraction temperature on the polyphenols extraction.

Fig. 1 show the interaction between the independent variables and their effects on the polyphenols extraction. It is worth to note that the highest TPC was observed at intermediate β-CD concentrations (X_1 variable) and intermediate extraction temperatures (X_3 variable) at constant ultrasonic treatment time of 5.9 min (Fig. 1A), according to the regression analysis (Eq. 5). The combination of these variables (X_1X_3) showed a synergistic effect on the polyphenols extraction, however the increased in β-CD concentration at a medium extraction temperatures led to an increase in the total polyphenolic contents.

The total polyphenol contents increased with the ultrasonic treatment (up to 15 min) at medium extraction temperatures (Fig. 1B) using constant concentration β-CD solution (15 mM). β-CD concentration (X_1) and the ultrasonic treatment time (X_2 variable) had lower effect when the temperature remained constant at 36.6 °C, as indicated by the obtained plane surface (Fig. 1C).

While high extraction temperature may increase the polyphenols yield due to an increase of solubility and diffusion of these compounds, those conditions may promote their degradation, thus reducing the global antioxidant capacity (Singh, Siddiq, Greiby, & Dolan, 2013).

β-cyclodextrin is commonly used as a protecting agent of sensible compounds and for increasing the solubility of the included ligands (Chao, Wang, Zhao, Zhang, & Zhang, 2012; Loftsson & Duchêne, 2007). The present results show that higher concentrations of β-cyclodextrin associated with ultrasound

ACCEPTED MANUSCRIPT

assisted extraction allows higher temperatures to be applied in order to increase the polyphenols extraction yield due probably to the protecting effect of their complexation (Gao et al., 2016).

3.1.2. Response surface analysis of free radical scavenging by DPPH• assay

Table 2 shows the results of applying the quadratic polynomial equation for antioxidant activity and extraction parameters (β-CD concentration, ultrasonic treatment time and extraction temperature). The acceptable regression value (R^2 = 0.9393) proved the reliability of the model. The generated equation in coded level, taking account only the significant terms for the antioxidant activity of thyme extracts, is given below:

$$Y_2\ (\mathrm{DPPH, mg\ GAE/mL}) = 14.27 - 1.37X_2 - 1.77X_3 + 0.60X_1X_2 - 0.86X_1X_3 - 2.14X_3^2 \quad (6)$$

The analysis of the applied model showed that the variables X_2, X_3, X_1X_2, X_1X_3 and X_3^2 were the most significant parameters on the combined factor extraction of the antioxidant activity of thyme extracts. The combination of variables X_1X_2 and X_1X_3 presented a synergistic effect on the antioxidant activity while X_1, X_2X_3, X_1^2 and X_2^2 had less effect on it.

Fig. 2 shows the Response surface plots obtained for the relationship between the studied extraction variables and the antioxidant activity (measured as DPPH• degradation). Fig. 2A represented the mutual interaction between β-CD concentration and extraction temperature and their effect on the antioxidant activity, when ultrasonic treatment time remained constant 5.9 min. Antiradical activity increased as the increased extraction temperature at low β-CD concentration. The highest DPPH• degradation was observed at low β-CD concentration (2.5 mM) and at high extraction temperature (44 °C). Fig. 2B shows the relationship between extraction temperature and ultrasonic treatment time when β-CD concentration remained constant at 15 mM. The antirradical activity increased with increasing medium extraction temperatures (X_3) and it was independent on the time of ultrasonication (X_2), according to the regression analysis (Eq. 6). The combination of these variables (X_2X_3) showed a no-synergistic effect on the antioxidant activity. However, the heating treatment at temperatures higher than 44 °C reduced the antirradical activity probably due to the degradation of bioactive compounds present on the thyme extracts. The antioxidant activity at constant temperature (36.6 °C), decreased with increasing of ultrasonic treatment time (X_2) and β-CD concentration (X_1) (Fig. 2C). Fig. 2 (A, B and C) shows that the antioxidant capacity was affected by increasing the time of the ultrasound treatment and extraction

ACCEPTED MANUSCRIPT

temperatures probably as a result of the lability of bioactive compounds in the extracts (Parmar et al., 2015).

3.1.3. Response surface analysis of Ferric reducing/antioxidant power (FRAP)

The results shown in Table 2 indicate that the ferric reducing/antioxidant power and the extraction parameters followed a quadratic relationship, with a good regression coefficient (R^2 = 0.9862).

The quadratic polynomial equation in code level taking account only significant terms of the ferric reducing/antioxidant power of thyme extracts is given below:

$$Y_3\ (\text{FRAP, mg GAE/mL}) = 3.15 + 0.42X_1 + 0.070X_2 + 0.47X_3 - 0.38X_1X_2 - 0.28X_1X_3 - 0.18X_2X_3 - 0.29X_1^2 - 0.81X_2^2 - 0.63X_3^2 \quad (7)$$

For ferric reducing/antioxidant power extraction, all the independent variables were significant likewise, the combination of variables X_1X_2, X_1X_3 and X_2X_3 showed a synergistic effect.

In Fig. 3 the relationship between the ferric reducing/antioxidant power and the extraction variables studied (β-CD concentration, ultrasonic treatment time and temperature) was represented. The ferric reducing/antioxidant power (FRAP) of the thyme extract increased using high concentrated β-CD solutions at medium temperatures and constant ultrasonic treatment time (5.9 min) (Fig. 3A). FRAP also increased using high concentrated β-CD solutions at medium ultrasonic treatment time when the temperature remained constant at 36.6 °C (Fig. 3B). According to these results, Fig. 3C shows that ferric reducing/antioxidant power increased at medium ultrasonic treatment time and at medium temperature using fixed β-CD concentration solution the 15 mM. These experimental results confirmed the validity of the applied model for predicting ferric reducing/antioxidant power (Eq. 7). The highest ferric reducing/antioxidant power was obtained using a solution of β-CD 7.5 mM, at 35 °C with a sonication time of 7.7 min, as previously reported in Table 1.

It can be concluded that the presence of β-CD, the time of ultrasonication and the temperature significantly affected the extraction efficiency of the analytes. The different responses of the polyphenol compounds contents, the antioxidant activity and the ferric reducing/antioxidant power towards the studied extraction variables can be attributted to the different nature of the chemical reactions involved in their determination and to other bioactive compounds present in the extracts. The radical scavenging activity determined by the DPPH• assay and the reducing power determined by FRAP, allow to measure

ACCEPTED MANUSCRIPT

the total antioxidant activity through different mechanisms: the first acts by scavenging free radicals, the second by promoting a reducing environment, and thus inhibiting oxidative reactions (Sánchez-Moreno, C., A. Larrauri, J., & Saura-Calixto, 1999; Jones et al., 2017). Therefore, a direct relationship between these two parameters can not always be expected (Santos, Buera, & Mazzobre, 2012). It is noteworthy that the conditions of maximum the ferric reducing/antioxidant power and those for the maximum polyphenol contents are coincident probably due to the reducing power of polyphenols.

3.2. Optimum of extraction craft parameters

3.2.1. Desirability function approach

The desirability function, applied to a numerical optimization, assigns a score to a set of responses and chooses factors settings in order to maximize that score. This approach allows to obtain the combined values of all optimal variables in order to maximize the responses, while satisfying the restrictions of the model.

The overall desirability function was used to analyze the operational conditions that lead to the optimized extraction process of thyme bioactive compounds that maximized the responses variables. The maximum global desirability function (D = 0.875) was reached when the optimized process conditions were β-CD concentration of 15 mM, 5.90 min of ultrasonic treatment time and an extraction temperature at 36.6 °C. Thus, the use these experimental conditions would result in the obtaining of extracts with predicted concentration of total polyphenolic contents by FC (189.3 mg GAE/mL), antioxidant activity by DPPH• (14.8 mg GAE/mL) and ferric reducing/antioxidant power by FRAP (3.3 mg GAE/mL).

3.2.2. Experimental validation of optimized conditions

The experimental values were compared with the predicted ones using the percentage difference (PD) in order to verify the validity of the model. The differences between the predicted optimal values and the experimental ones were of 6.1 % for TPC, 4.7 % for antioxidant capacity and 2.4 % for the ferric reducing/antioxidant power.

The percentage differences (2.4 to 6.1 %) are considered low in the desired region where the responses were maximized. As reported by Rezende et al. (2017) these results prove the utility and validity of using an experimental planning as RSM and the desirability function approach to optimize multiple responses variables. This model allow the study of the optimized experimental conditions in order to obtain higher extraction yields of bioactive compounds with higher thyme extract (ThyE) antioxidant activity

3.3. Effectiveness of the thyme extracts on non-enzymatic browning inhibition

ACCEPTED MANUSCRIPT

Once the thyme extraction conditions predicted by the validated RSM model for the maximum TPC and antioxidant/reducing capacities were obtained, the antibrowning and potentially anti-glycant capacity of ThyE were assessed.

The non-enzymatic browning reaction goes through three major stages (early, intermediate and final) to produce BPs (Zhou, Li, & Yu, 2016). The UV absorption at 420 nm is usually used to analyzed the production of colored browning final products (Wang, Bao, & Chen, 2013; Hong, Meng, & Lu, 2015; Rangsansarid, Cheetangdee, Kinoshita, & Fukuda, 2008; Yu, Zhou, & Yang, 2017). The changes in the absorption at 420 nm can reflect the browning rate on the non-enzymatic browning reaction and can be used to study the kinetics of the reaction (Yu et al., 2017).

The residual absorbance of the ThyE was evaluated through absorption at 420 nm of control systems of GLU+ThyE and BSA+ThyE (3 % v/v). Both systems presented constant absorbance values of nearly 0.95 ± 0.08 and 0.99 ± 0.05 respectively, during all experimental times at all the assayed temperatures. These values were considered for normalizing the values obtained in all the experiments.

The normalized absorbances at 420 nm for the control systems BSA+GLU and BSA+GLU+β-CD and for thyme extract containing systems: BSA+GLU+ThyE at different reaction times and temperatures are presented in Fig. 4A and B. BSA+GLU and BSA+GLU+β-CD reaction models had similar absorption patterns at all conditions, indicating that the presence of the β-CD in the solution did not have an effect on the BPs production. As β-CD in solution did not affect the browning reaction, the difference in the absorption was attributed to the ThyE presence. The increase of temperature and reaction time increased the BPs production in control and in ThyE systems likewise the production of BPs decreased in presence of ThyE, this effect being mainly noticed at 90 °C. Other authors have reported that the bioactive compounds present in natural extracts can interact with reactants and products of Maillard reaction changing its kinetics (Yu et al., 2017). Particularly thymol, one of the major active compound of thyme, exerted its antimicrobial action through binding to membrane proteins by hydrophobic bonding and hydrogen bonding, thus changing the permeability of the membranes (Burt, 2004). This type of non-covalent interactions between bioactive compounds and proteins can probably explain the inhibition of BPs production in presence of ThyE.

The production of BPs vs reaction time fitted zero order reaction kinetics, consistent with data reported previously by other authors for similar systems, but with different amino acids and sugars (Rangsansarid et al., 2008; Yu et al., 2017). Therefore, the temperature dependence of the reaction constant was

ACCEPTED MANUSCRIPT

determined using the Arrhenius equation as described above. Fig. 4C shows the lineal representation of Arrhenius equation where the logarithmic dependence of the rate constant (ln *k*) of the browning production as a function of the inverse of the absolute temperature (1/T) follows a linear dependence (R^2 > 0.90) for BSA+GLU and BSA+GLU+β-CD control systems and for GLU+BSA+ThyE system.

The linear fitting obtain was Y = -24126 X + 62.36 (R^2 = 0.9901); Y = -25611 X + 66.32 (R^2 = 0.9977) and Y = -12268 X + 27.91 (R^2 = 0.9010) for BSA+GLU; BSA+GLU+β-CD and BSA+GLU+ThyE systems, respectively. The E_a calculated from the slope of each lineal fitting, was used to describe the dependence of the non-enzymatic browning reaction with temperature. The E_a values for the formation of BPs from BSA+GLU; BSA+GLU+β-CD and BSA+GLU+ThyE systems, at 70-90 °C were 201 ± 6 kJ/mol; 213 ± 7 kJ/mol and 103 ± 3 kJ/mol, respectively. The E_a values of the control systems with and without β-CD presented not significant differences (*p*-value <0.001), as previously discussed (Fig. 4A). The ThyE affected the kinetics of BPs decreasing the activation energy, which indicated lower temperature dependence. Consequently, the relative BPs decrease was manifested at high temperatures (Fig. 4C). The chemistry underlying the Maillard reaction is very complex and the global kinetics approach with E_a calculation proved to be a useful tool to study the ThyE effect on Maillard reaction.

4. Conclusions

β-CD-based ultrasound extraction of thyme bioactive compounds was optimized using the RSM. The fitted model was adequate due to the low absolute error value obtained by comparing predicted versus observed values. The amount of bioactive compounds extracted using optimized β-CD solutions was higher than the amount obtained using water as an extraction solvent. The optimal extraction conditions were achieved with a β-CD concentration of 15 mM, an ultrasonic treatment of 5.9 min and an extraction temperature of 36.6 °C. Thus, the use of the optimal experimental conditions resulted in an extract with a total polyphenolic contents of 189.28 mg GAE/mL, an antioxidant activity by DPPH• of 14.84 mg GAE/mL, and a ferric reducing/antioxidant power by FRAP of 3.32 mg GAE/mL. The effect on the Maillard reaction kinetic of the obtained thyme extract was investigated using a model system of BSA and glucose. β-CD used as an extraction solvent proved to increase the stability of the extracted antioxidant compounds and did not affected Maillard reaction development. The presence of ThyE greatly inhibited Maillard reaction at high temperatures. The inhibition data are consistent with the changes in the activation energy and, therefore on the temperature dependence. For the first time β-CD

ACCEPTED MANUSCRIPT

extraction of antioxidants from thyme was optimized and the potential application of the thyme-β-CD extract as an anti-browning agent was assessed. The obtained data are important for developing emerging green extraction technologies using ultrasound assisted technique associated to eco-friendly solvents. Furthermore, Maillard glycation reaction agents with inhibitory potential were assessed in thyme antioxidant extracts. These findings will benefit and promote the effective application of natural source additives in replacement of synthetic ones.

Conflict of interest

All authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica, ANPCyT, (PICT 2013-1331) and Universidad de Buenos Aires (UBACYT 20020130100443BA).

References

Assiri, A. M. A., Elbanna, K., Abulreesh, H. H., & Ramadan, M. F. (2016). Bioactive Compounds of Cold-pressed Thyme (Thymus vulgaris) Oil with Antioxidant and Antimicrobial Properties, *640*(8), 629–640.

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R., & Simal-Gándara, J. (2009). A review on the use of cyclodextrins in foods. *Food Hydrocolloids*, *23*(7), 1631–1640. http://doi.org/10.1016/j.foodhyd.2009.01.001

Astray, G., Mejuto, J. C., Morales, J., Rial-otero, R., & Simal-gándara, J. (2010). Factors controlling flavors binding constants to cyclodextrins and their applications in foods. *Food Research International*, *43*(4), 1212–1218. http://doi.org/10.1016/j.foodres.2010.02.017

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, *99*(1), 191–203. http://doi.org/10.1016/j.foodchem.2005.07.042

Barba, F. J., Zhu, Z., Koubaa, M., Sant'Ana, A. S., & Orlien, V. (2016). Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review.

ACCEPTED MANUSCRIPT

Trends in Food Science and Technology, *49*(January), 96–109. http://doi.org/10.1016/j.tifs.2016.01.006

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. http://doi.org/10.1006/abio.1996.0292

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, (94), 223–253.

Chaillou, L. L., & Nazareno, M. A. (2006). New method to determine antioxidant activity of polyphenols. *Journal of Agricultural and Food Chemistry*, *54*(22), 8397–8402. http://doi.org/10.1021/jf061729f

Chao, J., Wang, H., Zhao, W., Zhang, M., & Zhang, L. (2012). Investigation of the inclusion behavior of chlorogenic acid with hydroxypropyl-β-cyclodextrin. *International Journal of Biological Macromolecules*, *50*(1), 277–282. http://doi.org/10.1016/j.ijbiomac.2011.11.008

Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green Extraction of Natural Products : Concept and Principles, 8615–8627. http://doi.org/10.3390/ijms13078615

Chen, M., Zhao, Y., & Yu, S. (2015). Optimisation of ultrasonic-assisted extraction of phenolic compounds , antioxidants , and anthocyanins from sugar beet molasses. *FOOD CHEMISTRY*, *172*, 543–550. http://doi.org/10.1016/j.foodchem.2014.09.110

Deepak, V., Kalishwaralal, K., Ramkumarpandian, S., Babu, S. V., Senthilkumar, S. R., & Sangiliyandi, G. (2008). Bioresource Technology Optimization of media composition for Nattokinase production by Bacillus subtilis using response surface methodology, *99*, 8170–8174. http://doi.org/10.1016/j.biortech.2008.03.018

Derringer, G. & Suich, R. (1980). Simultaneous Optimization of Several Response Variables. *Journal of Quality Technology*, *12*(4), 214–219. http://doi.org/10.1017/CBO9781107415324.004

Gao, F., Zhou, T., Hu, Y., Lan, L., Vander, Y., Crommen, J., … Fan, G. (2016). Cyclodextrin-based ultrasonic-assisted microwave extraction and HPLC-PDA-ESI-ITMS n separation and identification of hydrophilic and hydrophobic components of Polygonum cuspidatum : A green , rapid and effective process, *80*, 59–69.

Hong, X., Meng, J., & Lu, R. R. (2015). Improvement of ACE inhibitory activity of casein hydrolysate by Maillard reaction with xylose. *Journal of the Science of Food and Agriculture*, *95*(1), 66–71. http://doi.org/10.1002/jsfa.6682

ACCEPTED MANUSCRIPT

Hussein, J., Teshale, C., & Mohammed, J. (2011). Assesment of the Antimicrobial Effects of Some Ethiopian Aromatic Spice and Herb Hydrosols. *International Journal of Pharmacology*, *7*(5), 635–640.

Jones, A., Pravadali-Cekic, S., Dennis, G. R., Bashir, R., Mahon, P. J., & Shalliker, R. A. (2017). Ferric reducing antioxidant potential (FRAP) of antioxidants using reaction flow chromatography. *Analytica Chimica Acta*, *967*, 93–101. http://doi.org/10.1016/j.aca.2017.02.032

Liu, Q., Meng, X., Li, Y., Zhao, C., Tang, G., & Li, H. (2017). Antibacterial and Antifungal Activities of Spices, 1–62. http://doi.org/10.3390/ijms18061283

Loftsson, T., & Duchˆ, D. (2007). Cyclodextrins and their pharmaceutical applications, *329*, 1–11. http://doi.org/10.1016/j.ijpharm.2006.10.044

Martín-diana, A. B., Rico, D., & Barry-ryan, C. (2008). Green tea extract as a natural antioxidant to extend the shelf-life of fresh-cut lettuce, *9*, 593–603. http://doi.org/10.1016/j.ifset.2008.04.001

Morimitsu, Y., Yoshida, K., Esaki, S., & Hirota, A. (1995). Protein Glycation Inhibitors from Thyme (*Thymus vulgaris*). *Bioscience, Biotechnology and Biochemistry*, *59*(11), 2018–2021. http://doi.org/10.1271/bbb.59.2018

Nipornram, S., Tochampa, W., Rattanatraiwong, P., & Singanusong, R. (2018). Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (Citrus reticulata Blanco cv. Sainampueng) peel. *Food Chemistry*, *241*(March 2017), 338–345. http://doi.org/10.1016/j.foodchem.2017.08.114

Parmar, I., Sharma, S., & Rupasinghe, H. P. V. (2015). Optimization of β-cyclodextrin-based flavonol extraction from apple pomace using response surface methodology. *Journal of Food Science and Technology*, *52*(4), 2202–2210. http://doi.org/10.1007/s13197-014-1282-1

Rakmai, J., Cheirsilp, B., Mejuto, J. C., Simal-Gándara, J., & Torrado-Agrasar, A. (2018). Antioxidant and antimicrobial properties of encapsulated guava leaf oil in hydroxypropyl-beta-cyclodextrin. *Industrial Crops and Products*, *111*(October 2017), 219–225. http://doi.org/10.1016/j.indcrop.2017.10.027

Rakmai, J., Cheirsilp, B., Mejuto, J. C., Torrado-Agrasar, A., & Simal-Gándara, J. (2017). Physico-chemical characterization and evaluation of bio-efficacies of black pepper essential oil encapsulated in hydroxypropyl-beta-cyclodextrin. *Food Hydrocolloids*, *65*, 157–164. http://doi.org/10.1016/j.foodhyd.2016.11.014

ACCEPTED MANUSCRIPT

Rangsansarid, J., Cheetangdee, N., Kinoshita, N., & Fukuda, K. (2008). Bovine Serum Albumin-Sugar Conjugates through the Maillard Reaction : Effects on Interfacial Behavior and Emulsifying Ability, *547*(10), 539–547.

Ratnasooriya, C. C., & Rupasinghe, H. P. V. (2012). Extraction of phenolic compounds from grapes and their pomace using b -cyclodextrin. *Food Chemistry*, *134*(2), 625–631. http://doi.org/10.1016/j.foodchem.2012.02.014

Rezende, Y. R. R. S., Nogueira, J. P., & Narain, N. (2017). Comparison and optimization of conventional and ultrasound assisted extraction for bioactive compounds and antioxidant activity from agro-industrial acerola (Malpighia emarginata DC) residue. *LWT - Food Science and Technology*, *85*, 158–169. http://doi.org/10.1016/j.lwt.2017.07.020

Sánchez-Moreno, C., A. Larrauri, J., & Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines , grape juices and related polyphenolic constituents, *32*, 407–412.

Santos, C., Buera, M. P., & Mazzobre, M. F. (2012). Influence of ligand structure and water interactions on the physical properties of b -cyclodextrins complexes. *Food Chemistry*, *132*(4), 2030–2036. http://doi.org/10.1016/j.foodchem.2011.12.044

Schlesier, K., Harwat, M., Böhm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research*, *36*(2), 177–187. http://doi.org/10.1080/10715760290006411

Singh, D., Siddiq, M., Greiby, I., & Dolan, K. D. (2013). Total phenolics , antioxidant activity , and functional properties of " Tommy Atkins " mango peel and kernel as affected by drying methods. *Food Chemistry*, *141*(3), 2649–2655. http://doi.org/10.1016/j.foodchem.2013.05.053

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*(1974), 152–178. http://doi.org/10.1016/S0076-6879(99)99017-1

Szente, L., & Szejtli, J. (2004). Cyclodextrins as food ingredients. *Trends in Food Science & Technology*, *15*(3-4), 137–142. http://doi.org/10.1016/j.tifs.2003.09.019

Torre, D. La, Elizabeth, J., Gassara, F., Kouassi, A. P., Brar, S. K., Belkacemi, K., … Kouassi, A. P. (2017). Spice use in food : Properties and benefits. *Critical Reviews in Food Science and Nutrition*, *57*(6), 1078–1088. http://doi.org/10.1080/10408398.2013.858235

ACCEPTED MANUSCRIPT

Wakte, P. S., Sachin, B. S., Patil, A. A., Mohato, D. M., Band, T. H., & Shinde, D. B. (2011). Optimization of microwave , ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from Curcuma longa. *Separation and Purification Technology*, *79*(1), 50–55. http://doi.org/10.1016/j.seppur.2011.03.010

Wang, W. Q., Bao, Y. H., & Chen, Y. (2013). Characteristics and antioxidant activity of water-soluble Maillard reaction products from interactions in a whey protein isolate and sugars system. *Food Chemistry*, *139*(1-4), 355–361. http://doi.org/10.1016/j.foodchem.2013.01.072

Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, *105*(3), 940–949. http://doi.org/10.1016/j.foodchem.2007.04.038

Yu, A. N., Zhou, Y. Y., & Yang, Y. N. (2017). Kinetics of browning and correlations between browning degree and pyrazine compounds in L-ascorbic acid/acidic amino acid model systems. *Food Chemistry*, *221*, 1678–1684. http://doi.org/10.1016/j.foodchem.2016.10.119

Zhou, Y., Li, Y., & Yu, A. (2016). The effects of reactants ratios , reaction temperatures and times on Maillard reaction products of the L-ascorbic acid / L-glutamic acid system, *36*(2), 268–274.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Values of independent variables and their code forms with their symbols employed in RSM for optimization of thyme extract through ultrasonic-assisted extraction. Total polyphenolic contents, ferric reducing/antioxidant power, and antioxidant activity of the thyme extract obtained under different conditions based on a Box-Behnken design for response surface analysis.

Run	Extraction conditions			Response variables		
	Code and decoded variables					
	X_1	X_2	X_3	Y_1 Total polyphenolic contents (TPC)	Y_2 Antioxidant activity (DPPH•)	Y_3 Ferric reducing/antioxidant power (FRAP)
	β-CD concentration (mM)	Ultrasonic treatment time (min)	Extraction temperature (°C)	(mg GAE/mL thyme extract)		
1	(0) 7.5	(0) 7.7	(0) 35	199.6 ± 0.3	13.3 ± 0.5	3.36 ± 0.04
2	(-1) 0	(1) 15	(0) 35	166.5 ± 0.9	12.7 ± 0.8	2.14 ± 0.04
3	(-1) 0	(0) 7.7	(-1) 20	126.8 ± 0.6	9.2 ± 0.3	1.08 ± 0.02
4	(1) 15	(0) 7.7	(-1) 20	169.8 ± 0.5	11.6 ± 0.8	2.5 ± 0.1
5	(-1) 0	(0) 7.7	(1) 50	180.2 ± 0.7	14.67 ± 0.05	2.54 ± 0.07
6	(0) 7.5	(1) 15	(1) 50	156.2 ± 0.9	12.21 ± 0.06	2.03 ± 0.04

ACCEPTED MANUSCRIPT

7	(-1) 0	(-1) 0.5	(0) 35	156.2 0.6	16.1 ± 0.2	1.13 ± 0.07
8	(0) 7.5	(-1) 0.5	(1) 50	165.4 ± 0.5	15.5 ± 0.4	2.35 ± 0.01
9	(0) 7.5	(0) 7.7	(0) 35	171.9 ± 0.8	14.6 ± 0.2	3.04 ± 0.03
10	(1) 15	(0) 7.7	(1) 50	159.0 ± 0.9	13.6 ± 0.4	2.83 ± 0.01
11	(0) 7.5	(1) 15	(-1) 20	132.83 ± 0.07	8.86 ± 0.06	1.43 ± 0.01
12	(1) 15	(-1) 0.5	(0) 35	177.6 ± 0.9	15.0 ± 0.4	2.72 ± 0.03
13	(0) 7.5	(-1) 0.5	(-1) 20	127.0 ± 0.4	12.1 ± 0.7	1.04 ± 0.05
14	(0) 7.5	(0) 7.7	(0) 35	172.6 ± 0.6	15.0 ± 0.4	3.06 ± 0.03
15	(1) 15	(1) 15	(0) 35	184.3 ± 0.6	13.93 ± 0.08	2.21 ± 0.05

All results are the means ± SD (n = 3).

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 2. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of extractions parameters.

Source	Total polyphenolic contents (R^2 = 0.8965)					Antioxidant activity (R^2 = 0.9393)					Ferric reducing/antioxidant power (R^2 = 0.9862)				
	SS	DF	MS	*F*-value	*p*-value	SS	DF	MS	*F*-value	*p*-value	SS	DF	MS	*F*-value	*p*-value
Model	16598.36	9	1844.26	33.67	< 0.0001	186.34	9	20.70	60.16	< 0.0001	26.21	9	2.91	235.98	< 0.0001
Lack of fit	405.73	3	135.24	2.86	0.0521	2.12	3	0.71	2.28	0.0979	0.077	3	0.026	2.32	0.0366*
Pure error	1511.55	32	47.24			9.92	32	0.31			0.26	32	0.008042		

SS, sum of squares. DF, degree of freedom. MS, mean square. Significance level = $p \leq 0.05$; * $p \leq 0.01$.

ACCEPTED MANUSCRIPT

Legends for figures

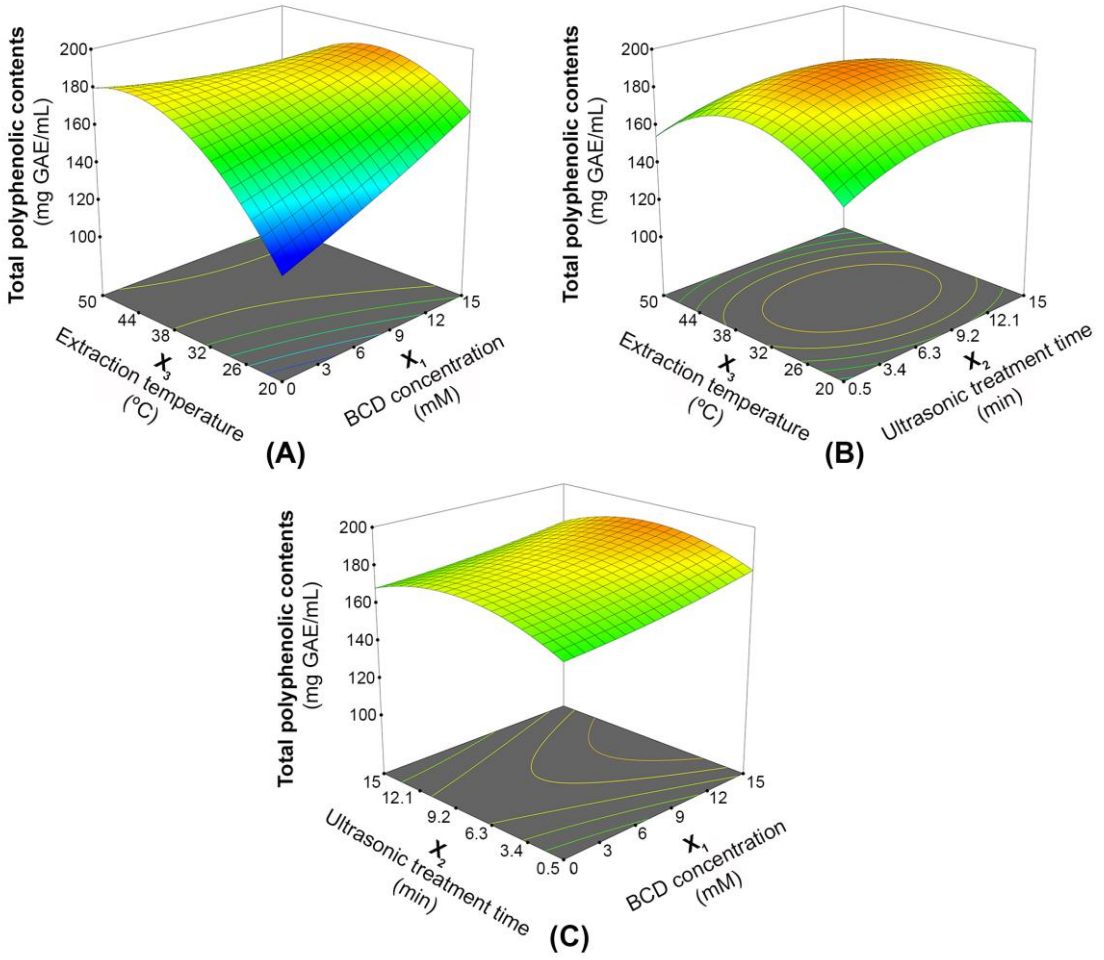
Fig. 1. Response surface plots of independent variables for the total polyphenolic contents of thyme extracts (TPC, mg GAE/mL) showing the maximum for the variables combination. TPC as a function of **(A)** extraction temperature and β-CD concentration using constant ultrasonic treatment time at 5.96 min; **(B)** temperature and ultrasonic treatment time using constant β-CD concentration 15 mM; **(C)** Ultrasonic treatment time and β-CD concentration using constant temperature at 36.60 °C.

Fig. 2. Response surface plots of independent variables for the antioxidant activity (AO), as DPPH radical degradation in mg GAE/mL, of thyme extracts. AO as a function of **(A)** extraction temperature and β-CD concentration using constant ultrasonic treatment time at 5.96 min; **(B)** temperature and ultrasonic treatment time using constant β-CD concentration 15 mM; **(C)** Ultrasonic treatment time and β-CD concentration using constant temperature at 36.60 °C.

Fig. 3. Response surface plots of independent variables for ferric reducing/antioxidant power (FRAP), in mg GAE/mL, of thyme extract showing the maximum for the of variables combination. FRAP as a function of **(A)** extraction temperature and β-CD concentration using constant ultrasonic treatment time at 5.96 min; **(B)** Ultrasonic treatment time and β-CD concentration using constant temperature at 36.6 °C; **(C)** temperature and ultrasonic treatment time using constant β-CD concentration 15mM.

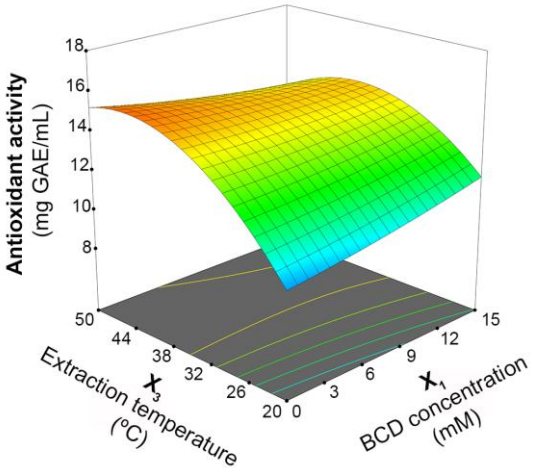
Fig. 4. (A) Absorbance at 420 nm of the control systems BSA+GLU (continuous line) and BSA+GLU+β-CD (dotted lines) as a function of the reaction times at different temperatures 70, 80 and 90 °C. (**B)** Absorbance at 420 nm of systems containing thyme extract (BSA+GLU+ThyE) in continuous line, and control systems (BSA+GLU+β-CD) in dotted line, at different reaction times and temperatures 70 (Inset), 80 and 90 °C. (**C)** Arrhenius plots for the control systems BSA+GLU and BSA+GLU+β-CD systems (dotted lines) and of systems with thyme (BSA+GLU+ThyE) in continuous line, ln *k*: logarithm of browning rate constants; 1/T: inverse of the absolute temperature.

The bars represent the standard deviation for each determination (n = 3), they are not observed when are smaller than the size of the symbol.

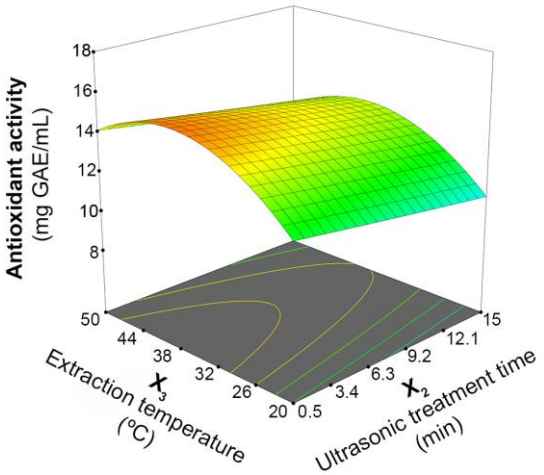

Total polyphenolic contents
(mg GAE/mL)
200
180
160
140
120
100
50
44
38
32
26
20
Extraction temperature
(°C)
X3
0
3
6
9
12
15
X1
BCD concentration
(mM)
(A)
Total polyphenolic contents
(mg GAE/mL)
200
180
160
140
120
100
50
44
38
32
26
20
Extraction temperature
(°C)
X3
0.5
3.4
6.3
9.2
12.1
15
X2
Ultrasonic treatment time
(min)
(B)
Total polyphenolic contents
(mg GAE/mL)
200
180
160
140
120
100
15
12.1
9.2
6.3
3.4
0.5
Ultrasonic treatment time
(min)
X2
0
3
6
9
12
15
X1
BCD concentration
(mM)
(C)

ACCEP

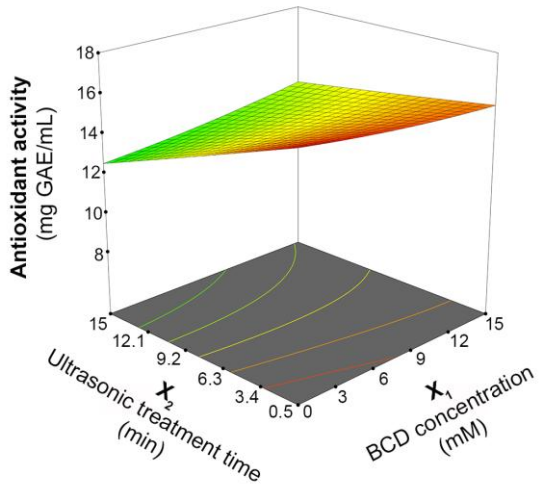
ACCEPTED MANUSCRIPT



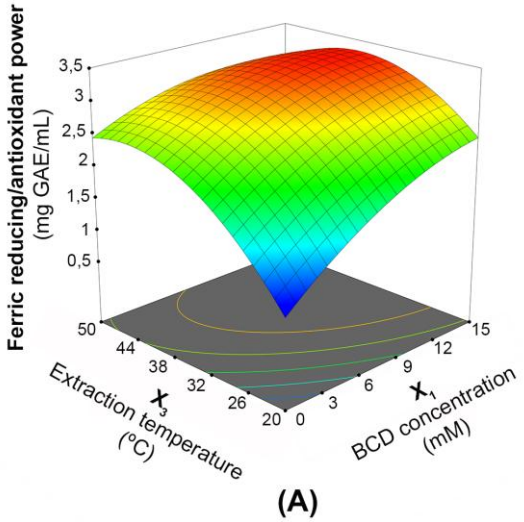
(A)



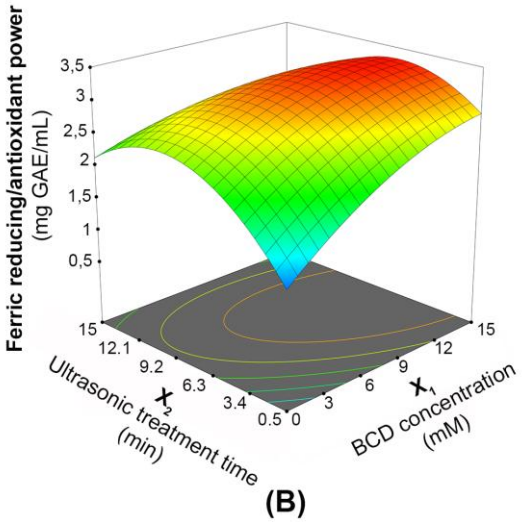
(B)



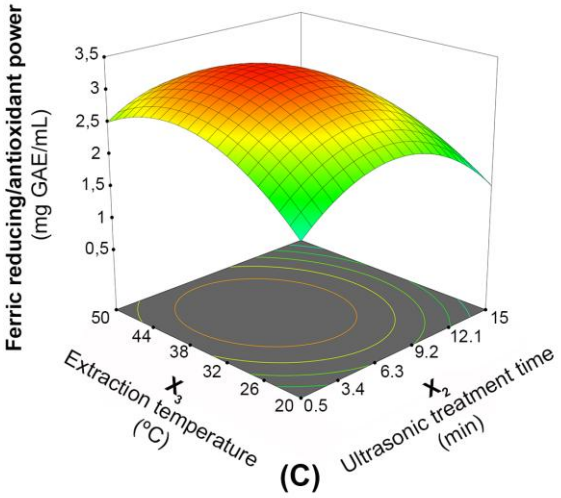
(C)


Ferric reducing/antioxidant power
(mg GAE/mL)
3,5
3
2,5
2
1,5
1
0,5
50
44
38
32
26
20
X_3
Extraction temperature
(°C)
0
3
6
9
12
15
X_1
BCD concentration
(mM)

(A)

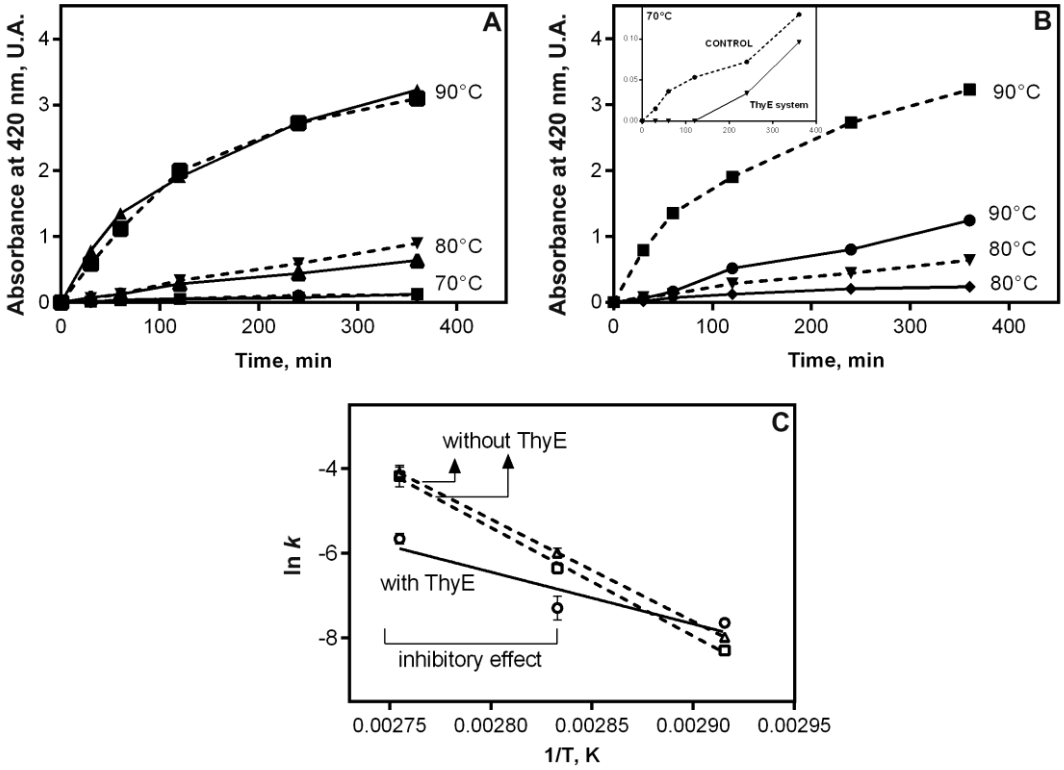

Ferric reducing/antioxidant power
(mg GAE/mL)
3,5
3
2,5
2
1,5
1
0,5
15
12.1
9.2
6.3
3.4
0.5
X_2
Ultrasonic treatment time
(min)
0
3
6
9
12
15
X_1
BCD concentration
(mM)

(B)


Ferric reducing/antioxidant power
(mg GAE/mL)
3,5
3
2,5
2
1,5
1
0,5
50
44
38
32
26
20
X_3
Extraction temperature
(°C)
0.5
3.4
6.3
9.2
12.1
15
X_2
Ultrasonic treatment time
(min)

(C)

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

- Thyme extract was obtained using β-cyclodextrin based ultrasound extraction
- Response surface methodology allowed optimizing extraction variables
- Optimized extraction variables were β-ciclodextrin, ultrasound time and temperature
- Thyme extract exhibited high antioxidant capacity and polyphenols content
- The extract demonstrated to inhibit the production of Maillard browning products

ACCEPTED MANUSCRIPT